Mechanism of Action of Tyrosine Kinase Inhibitors in Colon Cancer Disease: An In Silico Approach

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Introduction:

Cancer is a complex and heterogeneous disease characterized by uncontrolled cell growth and proliferation. At the molecular level, cancer is caused by a series of genetic mutations, Chromosomal abnormalities, Dysregulation of signaling pathways and epigenetic changes that disrupt the normal regulation of cell growth and division. These changes can lead to uncontrolled proliferation of cells. Cancer ranks as a leading cause of death and an important barrier to increasing life expectancy in every country of the world. According to estimates from the World Health Organization (WHO) in 2019, cancer is the first or second leading cause of death [1-2]. Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020, or nearly one in six deaths. The most common cancers are breast, lung, colon and rectum and prostate cancers [03].

Tyrosine kinases are a family of cell surface receptors, which act as receptors for growth factors, hormones, cytokines, neurotrophic factors and other extracellular signaling molecules. Receptor Tyrosine Kinases (RTKs) mediate key signaling pathways that are involved in cell proliferation, differentiation, survival and cell migration [4]. The RTK family comprises several subfamilies which include, among others, epidermal growth factor receptors (EGFRs), fibroblast growth factor receptors (FGFRs), insulin and insulin-like growth factor receptors (IR and IGFR), platelet-derived growth factor receptors (PDGFRs), vascular endothelial growth factor receptors (VEGFRs), hepatocyte growth factor receptors (HGFRs), and proto-oncogene c-KIT [5,6].

Receptor Tyrosine Kinases (RTKs) are responsible for adding a phosphate group to specific tyrosine residues on target proteins, a process known as phosphorylation. This phosphorylation event acts as a molecular switch, activating or deactivating downstream signaling pathways involved in cell signaling, signal transduction, cell growth and proliferation, cell differentiation and development, and survival. Mutation, overexpression, signaling pathway deregulation and dysfunction of tyrosine kinase receptors lead to the development of malignancy; thus, they are considered one of the important targets for the development of anti-cancer molecules.

The tyrosine kinase family is majorly divided into two classes; receptor and non-receptor tyrosine kinase. Both of the classes have an important role in the development of tumour cells. In the human genome, ninety tyrosine kinases have been identified, including fifty-six receptor tyrosine kinases and thirty-two cellular tyrosine kinases [07]. Tyrosine kinase can be broadly classified into two main classes based on their structure and activation mechanisms:

1. Receptor Tyrosine Kinases (RTKs):

Receptor tyrosine kinases are transmembrane proteins that span the cell membrane and have both an extracellular ligand-binding domain and an intracellular tyrosine kinase domain. Upon ligand binding to their extracellular domain, they undergo dimerization or oligomerization, which activates their intracellular tyrosine kinase activity. This autophosphorylation on specific tyrosine residues initiates downstream signaling cascades, leading to various cellular responses.

Examples of receptor tyrosine kinases include:
- Epidermal Growth Factor Receptor (EGFR)
- Platelet-Derived Growth Factor Receptor (PDGFR)
- Insulin Receptor (IR)
- Fibroblast Growth Factor Receptor (FGFR)
- Vascular Endothelial Growth Factor Receptor (VEGFR)

2. Non-Receptor Tyrosine Kinases:

Non-receptor tyrosine kinases are located in the cytoplasm or associated with the cell membrane but lack the extracellular ligand-binding domain of RTKs. Instead, they are activated by other proteins or signaling events. Non-receptor tyrosine kinases play crucial roles in intracellular signaling and regulate various cellular processes.

Examples of non-receptor tyrosine kinases include:
- Src family kinases (e.g., Src, Yes, Fyn)
- Janus Kinases (JAKs)
- Abl kinase
- Bcr-Abl kinase (a fusion protein resulting from a genetic abnormality in chronic myeloid leukemia)
- Bruton's tyrosine kinase (BTK)
- Tyrosine-protein kinase SYK

Both classes of tyrosine kinases are essential for cellular signal transduction and play critical roles in the regulation of cell growth, differentiation, survival, and other physiological processes. Dysregulation of tyrosine kinases can lead to various diseases, including cancer, autoimmune disorders, and metabolic disorders. Consequently, pharmaceutical research has focused on developing targeted therapies, such as tyrosine kinase inhibitors (TKIs), to treat diseases associated with aberrant tyrosine kinase activity.

Currently, more than 40 FDA-approved tyrosine kinase inhibitors are used to treat various types of cancers. Tyrosine kinase inhibitors mainly block the phosphorylation of tyrosine residue of the corresponding kinase substrate, so activation of downstream signalling pathways can be inhibited. The promising results of tyrosine kinase inhibitors in solid tumours have revolutionized oncology research. Tyrosine kinases are a diverse group of enzymes that play crucial roles in cell signaling and regulation. They are classified into two main categories based on their location and activation:

Tyrosine kinases play a crucial role in cellular signaling pathways that regulate cell growth, survival, and differentiation. Dysregulation of tyrosine kinase activity is frequently observed in various types of cancers, making them attractive targets for therapeutic intervention. Tyrosine

kinase inhibitors (TKIs) have emerged as a promising class of drugs that specifically target and inhibit the activity of these kinases, thereby disrupting aberrant signaling pathways and inhibiting cancer cell growth. This study aims to investigate the mechanism of action of TKIs in selected cancer diseases using an in Silico approach.

VEGFR Family:

VEGFR family consists of three receptors i.e., VEGFR1, VEGFR2 and VEGFR3 which belongs to the tyrosine kinase family receptors (transmembrane proteins). All tyrosine kinase receptors are highly homologous in their architecture and includes immunoglobulin-like domains (extracellular domain), transmembrane helix domain, and cytoplasmic domain which consists of juxtamembrane and kinase domain regions (KDR).

VEGFR2 (Vascular endothelial growth factor receptor 2) is one of the two tyrosine kinase receptors involved in angiogenesis. When activated by its ligand VEGF, VEGFR2 promotes neighbouring vessel formation to facilitate the delivery of growth factors, nutrients and oxygen for cancer proliferation, migration, metastasis and survival [08]. VEGF and VEGFR2 mediated angiogenesis contributes to the aggressive natures and leads to high mortality rate in gastric cancer, which is the third leading cause of cancer deaths worldwide [09].

EGFR (epidermal growth factor receptor, also known as ERBB1 and HER1) is a gene that encodes for the epidermal growth factor receptor protein. Missense mutations, deletions, and insertions are observed in cancers such as lung cancer and glioblastoma. Activating EGFR mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways (PMID: 15284455).

EGFR is altered in 6.83% of all cancers with lung adenocarcinoma, conventional glioblastoma multiforme, glioblastoma, breast invasive ductal carcinoma, and colon adenocarcinoma having the greatest prevalence of alterations [10].

Literature review:

Cancer is a class of diseases in which a group of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasize. Development of cancer is generally considered a multistep process driven by carcinogen-induced genetic and epigenetic damage in susceptible cells, which as a result gain selective growth damage. Subsequently the cells may undergo clonal expansion as the result of activation of protooncogenes and/or inactivation of tumor suppressor genes (Kowali et al., 2003; Barrett et al., 1993; Harris et al., 1991).

Manash K. Paul et al (2004) was reported Tyrosine kinases are important mediators of the signaling cascade, determining key roles in diverse biological processes like growth, differentiation, metabolism and apoptosis in response to external and internal stimuli. Recent advances have implicated the role of tyrosine kinases in the pathophysiology of cancer. Though their activity is tightly regulated in normal cells, they may acquire transforming functions due to mutations, overexpression and autocrine paracrine stimulation, leading to malignancy. Constitutive oncogenic activation in cancer cells can be blocked by selective tyrosine kinase inhibitors.

Receptor tyrosine kinases (RTKs) play an important role in a variety of cellular processes including growth, motility, differentiation, and metabolism. As such, dysregulation of RTK signaling leads to an assortment of human diseases, most notably, cancers. Recent large-scale genomic studies have revealed the presence of various alterations in the genes encoding RTKs such as EGFR, HER2/ErbB2, and MET, amongst many others. Abnormal RTK activation in human cancers is mediated by four principal mechanisms: gain-of-function mutations, genomic amplification, chromosomal rearrangements, and / or autocrine activation (Zhenfang Du et al 2018).

Hoff P. M. et al (2012) has identified VEGFR-2 was the fundamental regulator of VEGF-driven responses in endothelial cells and can control proliferation, differentiation, and microvascular permeability. Over and above, it has shown to be a prerequisite signal transducer in both physiologic and pathologic angiogenesis. VEGFR-2 was overexpressed in several malignancies, including hepatocellular carcinoma, breast, colorectal, ovarian and thyroid cancer, melanoma and medulloblastoma. Therefore, VEGFR-2 has been identified as an excellent therapeutic target for the production of novel anticancer agents.

Angiogenesis was the procedure of formation of new blood vessels by regulating the chemical signals such as VEGF, which binds with the receptors on the surface of endothelial cells. VEGF receptors are classified into three types: VEGFR1, VEGFR2 and VEGFR3 respectively (Mustonen T et al.).

The vascular epithelial growth factor (VEGF), interacting with the tyrosine kinase receptor VEGFR-2 on endothelial cells, exerts its proangiogenic activity. Hence, targeting the VEGFR-2 signaling is considered a promising strategy to inhibit angiogenesis and thus cancer treatment. The bioactive compounds of R. serpentina, selected based on their physicochemical properties and drug-likeness, were docked against the human VEGFR-2 molecular target using the AutoDock 4.2 software. revealed the possible constituents of R. serpentina that can potentially bind with and inhibit VEGFR-2. This study further provides structural insights into the possible modes of interaction of the ligands with the target (Adel M. Abuzenadah et al.).

Objective:

- Comparative docking studies of available inhibitors with targeted protein and selection of best ligand.
- Screening or designing of novel potent tyrosine kinase inhibitors.
- To calculate the binding free energy of docked conformers (Gibbs free energy).
- Selection of best ligands based on various evaluations criteria.
- To predict the ADMET properties of selected compounds.

Methodology:

In this study, an in silico approach will be employed to analyze the mechanism of action of TKIs in specific cancer diseases. The following steps will be undertaken:

1. Selection of cancer diseases: A set of representative cancer diseases will be chosen for analysis based on their high prevalence, available TKI therapies, and relevance to tyrosine kinase dysregulation.

2. Tyrosine kinase targets: The tyrosine kinases that are known to be implicated in the selected cancer diseases will be identified through a comprehensive literature review and database mining. Now identify specific tyrosine kinases that play a critical role in the growth and progression of cancer cells. This involves extensive research to understand the molecular mechanisms driving the cancer and identifying the key signaling pathways and tyrosine kinases involved.

3. In silico modeling and docking studies: Computational models of the selected tyrosine kinases will be constructed based on available crystal structures or homology modeling techniques. Docking studies will be performed to investigate the binding interactions between the tyrosine kinase inhibitors and their respective targets. Molecular docking simulations are performed to predict the binding mode and binding affinity of the selected ligands with the target tyrosine kinase. Docking algorithms calculate the optimal orientation and conformation of the ligands within the active site of the kinase.

The docked ligands are scored based on their predicted binding affinity and other interactions with the target. This ranking helps prioritize the most promising compounds for further investigation.

4. Quantitative Structure-Activity Relationship (QSAR) Analysis: Through computational QSAR analysis, modifications are made to the promising ligands to optimize their potency and selectivity. This involves exploring chemical modifications that enhance the ligand's interactions with the target while minimizing interactions with other off-target proteins.

5. Analysis of signaling pathways: The downstream signaling pathways regulated by the selected tyrosine kinases will be analyzed using bioinformatics tools and databases. The effects of TKIs on these signaling pathways will be explored, focusing on the inhibition of key oncogenic signaling cascades.

6. ADMET Prediction: Absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the optimized compounds are predicted using computational models. This helps identify potential issues related to drug-like properties and toxicity.

The work will involve state of the art Chemoinformatics and bioinformatics techniques and modeling platforms. The docking protocol involved four main steps as mentioned below:

1) **Receptor preparation:**
2) **Ligand preparation:**
3) **Docking using a search algorithm:**
4) **Analysis of the binding conformation**

Importance of study:

Innovative targeted therapy is required to improved patient outcomes, overcoming resistance, reduced toxicity with shorten time period and reduced failure rate in Clinical phase to overcome the relapse, resistance and global burden of cancer.

Chapter scheme of the proposed research work: It is proposed that the thesis will be divided into three chapters with the following contents.

YEAR	TARGETS TO BE ACHIEVED
1	Brief introduction to tyrosine kinases in general and VEGFR in particular and status of present work with respect to literature.
2	Methodology used depicted in methodology section, online and proprietary software and databases which will be used for the above mentioned work.
3	Analyzing the obtained results and their validation. Submission of final thesis.

Expected Results:

The in silico analysis is expected to provide insights into the mechanism of action of TKIs in selected cancer diseases. The results will elucidate the binding interactions between TKIs and their target kinases, shed light on the downstream signaling pathways affected by TKI treatment, and identify potential mechanisms of drug resistance. This knowledge will contribute to a better understanding of the therapeutic efficacy of targeted protein or enzyme and limitations of TKIs in cancer treatment, ultimately aiding the development of more effective strategies to combat these diseases.

Conclusion:

This study proposes an in silico approach to investigate the mechanism of action of TKIs in selected cancer diseases. The findings will enhance our understanding of the molecular basis of TKI-mediated cancer therapy and provide valuable information for the development of personalized treatment strategies targeting specific tyrosine kinase-driven cancers. The results may contribute to the optimization of TKI therapy and the identification of novel therapeutic targets for improved cancer management.

References:

1) Bray F, Laversanne M, Weiderpass E, Soerjomataram I. The ever-increasing importance of cancer as a leading cause of premature death worldwide. Cancer. In press
2) World Health Organization (WHO). Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. WHO; 2020.
3) Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory: Cancer Today. Lyon: International Agency for Research on Cancer; 2020 (https://gco.iarc.fr/today, accessed February 2021).
4) Lemmon, M.A.; Schlessinger, J. Cell signaling by receptor tyrosine kinases. Cell 2010, 141, 1117–1134.
5) Schlessinger, J. Cell signaling by receptor tyrosine kinases. Cell 2000, 103, 211–225.
6) Hubbard, S.R. Juxtamembrane autoinhibition in receptor tyrosine kinases. Nat. Rev. Mol. Cell Biol. 2004, 5, 464–471.
7) Broekman, F., Giovannetti, E. & Peters, G. J. Tyrosine kinase inhibitors: multi-targeted or single-targeted? World J. Clin. Oncol. 2, 80 (2011).
8) Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol. 2016;17:611–25.
9) Yu J, Zhang Y, Leung LH, Liu L, Yang F, Yao X. Efficacy and safety of angiogenesis inhibitors in advanced gastric cancer: a systematic review and meta-analysis. J Hematol Oncol. 2016;9:111.
10) The AACR Project GENIE Consortium. AACR Project GENIE: powering precision medicine through an international consortium. Cancer Discovery. 2017;7(8):818-831. Dataset Version 8. This dataset does not represent the totality of the genetic landscape; see paper for more information.
11) Kovalli, G; Shiff, S.; Telang, N.; Das, K.; Kohgo, Y.; Narayan, S. and Li, H. (2003), 'Carcinogenesis: The more we seek to know the more we need to know – Challenges in the post Genomic Era' J Carcinog., 2, 1.
12) Barrett, J.C. (1993), 'Mechanism of multistep carcinogenesis and carcinogen risk assessment.' Environ Health Perspect., 100, 9-20.
13) Harris, C.C. (1991), 'Chemical and physical carcinogenesis: advances and perspectives for the 1990s' Cancer Res., 51, 5023-5044.
14) Manash K. Paul and Anup K. Mukhopadhyay Tyrosine kinase – Role and significance in Cancer Int. J. Med. Sci. 2004 1(2): 101-115
15) Du and Lovly Mechanisms of receptor tyrosine kinase activation in cancer Molecular Cancer (2018) 17:58 https://doi.org/10.1186/s12943-018-0782-4
16) Hoff P. M. Machado K. K. Role of angiogenesis in the pathogenesis of cancer. *Cancer Treat. Rev.* 2012;38 (7):825–833.
17) Mustonen T et al. The Journal of cell biology 1995 129:895. [7538139]

18) Adel M. Abuzenadah et al. "Elucidating Antiangiogenic Potential of Rauwolfia serpentina: VEGFR-2 Targeting-Based Molecular Docking Study", Evidence-Based Complementary and Alternative Medicine, vol. 2022, Article ID 6224666, 10 pages, 2022.

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